

d) generating a Boltzmann probability matrix comprising a plurality of altered nucleated state sequences.

45. (New) A method according to claim 44 wherein an ensemble of protein backbone scaffolds are inputted.

46. (New) A method according to claim 44 further comprising ranking said altered nucleated state sequences.

47. (New) A method according to claim 44 further comprising synthesizing a plurality of said altered nucleated state sequences.--

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#### REMARKS

Claims 1-15 have been cancelled without prejudice or disclaimer.

Claims 18-47 are newly added. Claims 16-47 are now pending. An appendix of "PENDING CLAIMS" is attached for the Examiner's convenience.

Support for new claims 18-25 can be found in various places in the specification, for example at page 5, lines 18-22 and Figure 2.

Support for new claims 27-38 can be found throughout the specification, for example at page 3, lines 6-14; page 19, lines 25-33. Additional support can be found in original claims 1-14.

Support for new claims 29 and 46 can be found at page 3, lines 20-21 and page 5, lines 18-22.

Support for new claim 30 and 47 can be found at page 3, lines 20-21 and page 30, lines 21-32.

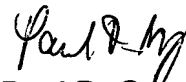
Support for new claims 44-45 can be found in various places in the specification, for example at page 20, lines 16-27; page 23, lines 8-43.

Consideration and allowance, being in order, are earnestly solicited. If the Examiner has further questions regarding the application, the undersigned would welcome a telephone call at (203) 327-4500.

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Date

Respectfully submitted,



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## **PENDING CLAIMS**

16. A method for optimizing simulation or scoring function parameters that utilizes comparisons between designed sequences and natural sequences, comprising the steps of:

- a) applying a protein design cycle to produce a variable protein sequence;
- b) comparing said variable protein sequence to at least one natural protein sequence and/or conformation;
- c) modifying said simulation or scoring function parameters to reflect said comparison.

17. A method for optimizing simulation or scoring function parameters that utilizes comparisons between designed sequences and natural sequences, comprising the steps of:

- a) applying a protein design cycle to produce an amino acid probability matrix;
- b) comparing said matrix to at least one natural protein sequence and/or conformation;
- c) modifying said simulation or scoring function parameters to reflect said comparison.

--18. (New) A method executed by a computer under the control of a program, said computer including a memory for storing said program, said method comprising the steps of:

- d) inputting a protein backbone scaffold;
  - e) applying a first protein design cycle comprising at least one heuristic component to said scaffold to generate at least a first variable sequence;
  - f) applying a second protein design cycle comprising at least one heuristic component to said scaffold to generate at least a second variable sequence; and
  - d) generating a probability matrix comprising at least said first and second variable sequences.
19. (New) A method according to claim 18 wherein said first and second protein design cycle comprise the same components.
20. (New) A method according to claim 18 wherein said first and second protein design cycle comprise different components.
21. (New) A method according to claim 18 wherein at least one of said heuristic components comprises a genetic algorithm.
22. (New) A method according to claim 18 wherein at least one of said heuristic components comprises a Monte Carlo algorithm.
27. (New) A method according to claim 21 wherein at least one of said first and second protein design cycles comprises a self consistent mean field theory (SCMF) algorithm.
28. (New) A method according to claim 22 wherein at least one

of said first and second protein design cycles comprises a self consistent mean field theory (SCMF) algorithm.

29. (New) A method according to claim 21 wherein at least one of said first and second protein design cycles comprises a dead end elimination (DEE) algorithm.

30. (New) A method according to claim 22 wherein at least one of said first and second protein design cycles comprises a dead end elimination (DEE) algorithm.

27. (New) A method according to claim 18 wherein said probability matrix comprises a summation of the variable sequences generated for each scaffold.

28. (New) A method according to claim 18 wherein said probability matrix comprises a recombination of some or all of the variable sequences generated for each scaffold.

29. (New) A method according to claim 18 further comprising ranking said variable sequences.

30. (New) A method according to claim 18 further comprising synthesizing a plurality of said sequences

31. (New) A method according to claim 18 further comprising recombining a plurality of said variable sequences to form additional variable sequences.

33. (New) A method executed by a computer under the control of a

program, said computer including a memory for storing said program, said method comprising the steps of:

- c) inputting an ensemble of protein backbone scaffolds;
- d) applying a protein design cycle to each of said scaffolds to generate at least one variable sequence; and
- c) generating a probability matrix comprising a plurality of the variable sequences generated in step b).

33. (New) A method according to claim 32 wherein said probability matrix comprises a summation of the variable sequences generated for each scaffold.

34. (New) A method according to claim 32 wherein said probability matrix comprises a recombination of some or all of the variable sequences generated for each scaffold.

35. (New) A method according to claim 32 wherein said protein design cycle comprises a genetic algorithm.

36. (New) A method according to claim 32 wherein said protein design cycle comprises a Monte Carlo algorithm.

37. (New) A method according to claim 32 wherein said protein design cycle comprises a self consistent mean field theory (SCMF) algorithm.

38. (New) A method according to claim 32 wherein said protein design cycle comprises a dead end elimination (DEE) algorithm.

39. (New) A method according to claim 32 wherein said ensemble comprises a family of naturally occurring proteins.
40. (New) A method according to claim 32 wherein said ensemble is generated by a Monte Carlo simulation.
41. (New) A method according to claim 32 further comprising ranking said variable sequences.
42. (New) A method according to claim 32 further comprising synthesizing a plurality of said sequences.
43. (New) A method according to claim 32 further comprising recombining a plurality of said variable sequences to form additional variable sequences.
45. (New) A method executed by a computer under the control of a program, said computer including a memory for storing said program, said method comprising the steps of:
- e) inputting at least one protein backbone scaffold;
  - f) applying a protein design cycle to generate at least a first variable nucleated state sequence;
  - g) sequentially altering in said nucleated state sequence a plurality of amino acids by testing a plurality of rotamers for each amino acid change and calculating the energy of each altered sequence; and

h) generating a Boltzmann probability matrix comprising a plurality of altered nucleated state sequences.

45. (New) A method according to claim 44 wherein an ensemble of protein backbone scaffolds are inputted.

46. (New) A method according to claim 44 further comprising ranking said altered nucleated state sequences.

47. (New) A method according to claim 44 further comprising synthesizing a plurality of said altered nucleated state sequences.--